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Hepatomegaly and fever at the time of neutrophil recovery revealing L-asparaginase toxicity in the treatment of acute lymphoblastic leukemia

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Patient: Male, 52
Final Diagnosis: L-asparaginase associated steatohepatitis and pulmonary *Pneumocystis*
Symptoms: Cholestasis • hepatomegaly
Medication: Corticosteroids • atovaquone • antioxidant therapy
Clinical Procedure: Liver biopsy
Specialty: Hematology • Infectious Disease • Hepatology

Objective: Challenging differential diagnosis

Background: L-asparaginase (L-aspa) is an important component of chemotherapy in acute lymphoblastic leukemia (ALL). Main adverse effects of L-aspa include allergic reactions, pancreatitis, thrombosis, and liver disturbances. L-aspa-associated steatohepatitis may be a life-threatening disorder but has very rarely been reported in the literature.

Case Report: ALL was diagnosed in a 52-year-old man with a history of cardiovascular disease and obesity. Chemotherapy combining daunorubicin, vincristine, cyclophosphamide, and L-aspa was initiated. At the time of neutrophil recovery, the patient developed hepatomegaly in the context of fever and cough. On day 25, after 6 injections of L-aspa, liver function tests showed elevated alkaline phosphatase and transaminases levels. Although pulmonary *Pneumocystis* was concomitantly diagnosed, biological hepatic disturbances were attributed to L-aspa-associated toxicity. A liver biopsy revealed severe diffuse micro- and macrovesicular steatosis affecting more than 50% of hepatocytes. Other causes of liver dysfunction were eliminated. L-aspa and other hepatotoxic treatments were stopped, and treatment with antioxidant therapy, atovaquone, and corticosteroids was initiated. The clinical outcome was rapidly favorable.

Conclusions: This case illustrates the necessity of carefully monitoring liver function test results in patients receiving L-aspa. In case of major increase of hepatic enzymes, a hepatic biopsy should rapidly be performed to exclude differential diagnosis in patients with prolonged neutropenia. L-aspa should be stopped and further administration definitively avoided. In the present case, the early administration of systemic corticosteroids as treatment of the concomitant *Pneumocystis* with hypoxemia could have participated to the favorable clinical evolution.

Keywords: cholestasis • acute lymphoblastic leukemia • hepatomegaly • steatosis • L-asparaginase

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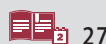
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Background

Two native *E. coli* and *Erwinia chrysanthemi* and a polyethylene glycol-conjugated (or -pegylated) *E. Coli* L-asparaginase (L-aspa) have made major contributions in the treatment outcome of childhood acute lymphoblastic leukemia (ALL) [1]. The enzyme hydrolyses asparagine (and glutamine) to aspartic acid and ammonia, while ALL cells cannot re-synthesize asparagines, which leads to their death [2]. Since the difference in the outcome in young adults when treated with a pediatric protocol and an adult protocol [3,4] (showing improved survival with pediatric protocol due to repetitive administration of several agents of which L-aspa) has been highlighted, cumulative L-aspa doses have been introduced in adults with pediatric-inspired therapy, resulting in improved outcome (2-year disease-free survival of 56%) [5]. The main adverse effects observed with L-aspa include anaphylaxis, pancreatitis, thrombovascular, or hemorrhagic disorders, and central nervous system disturbance [6]. It is generally believed that toxicities are more frequent in adults. L-aspa-associated severe diffuse steatosis has rarely been reported [7,8]. Prognosis is generally very poor due directly to the potential liver failure, and indirectly to the need to definitively stop the administration of one of the most important components in ALL chemotherapy.

We report here a case of severe L-aspa-associated steatohepatitis with a favorable immediate outcome. The initial clinical presentation was highly evocative of a hepatosplenic candidosis. This case thus illustrates the absolute necessity of rapidly implementing a systematic and invasive diagnosis strategy, including liver biopsy, in case of severe clinical and/or biological liver abnormalities during L-aspa therapy in ALL patients.

Case Report

A 52-year-old man with medical history marked by obesity (body weight 112 Kg, body mass index 33.7 kg/m²) and acute myocardial infarction (20 years ago), presented to our Institution in June 2013 with fever and dental pain. There was no more tobacco intoxication, nor alcohol consumption. Clinical examination was normal. Blood tests displayed hyperleukocytosis with 74% of circulating blasts, anemia, and thrombocytopenia. Hepatic tests showed moderate cytolysis and cholestasis: L-aspartate aminotransferase (AST) level at 59 IU/l, (N<40), L-alanine aminotransferase (ALT) at 44 IU/l, (N<56), gamma-glutamyltransferase (γ GT) at 69 IU/l, (N<42), alkaline phosphatase (APL) at 49 IU/l, (N<120), and total bilirubinemia (TBIL) at 35 μ mol/l, (N<20). An abdominal ultrasound was performed and revealed mild steatosis and absence of hepatomegaly. Because of fever, a piperacillin/tazobactam treatment (4 g/6 h) was rapidly initiated. Bone marrow aspirate confirmed the diagnosis of ALL with medullary infiltration by 90% of blastic

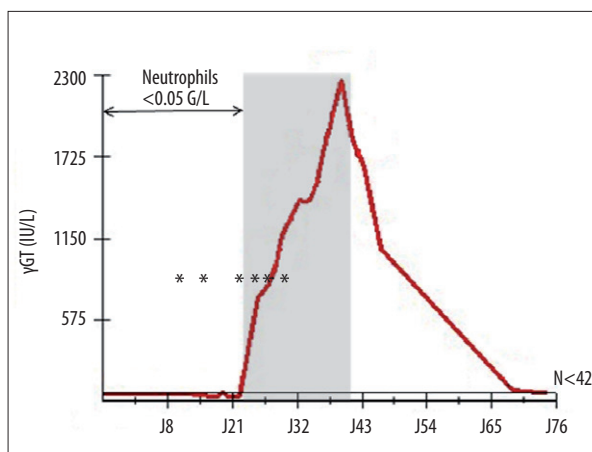


Figure 1. Clinical and biological course of L-asparaginase associated steatohepatitis. L-asparaginase (*) was prescribed from J8 (first injection) to J26 (6th and last injection). The patients experienced fever and biological inflammatory syndrome after neutrophils recovery from J22 to J37 (Grey zone). Atovaquone and corticosteroids for *Pneumocystis* treatment was started at J36. γ GT – gamma-glutamyltransferase.

cells immunophenotypically of B cell lineage (CD19+, CD22+, CD10–, CD20–). Molecular and cytogenetic analyses showed initial features of high-risk ALL (Ikaros deletion and t(4;11) with MLL rearrangement).

After a 1-week prephase with steroids, induction chemotherapy was started according to the GRAALL 2005 trial, including daunorubicin 50 mg/m²/day on days 1 to 3 and 30 mg/m²/day on days 15 and 16; vincristine 2 mg total dose on days 1, 8, 15, and 22; cyclophosphamide 750 mg/m²/day on days 1 and 15; and (theoretically) 8 injections of L-aspa 6000 IU/m²/day on days 8, 10, 12, 20, 22, 24, 26, and 28. Neuromeningeal prophylaxis was performed by intra-thecal injections of methotrexate (15 mg), methylprednisolone (40 mg), and cytarabine (40 mg). Granulocyte colony-stimulating factor (G-CSF) was planned from day 18 to the ultimate neutrophil recovery. During neutropenia, *Candida albicans* was isolated twice from systematic stool examination, without any digestive symptoms. At day 19, the patient presented abdominal tenderness, especially in the right upper quadrant, and hepatomegaly. While recovering neutrophils on day 22, he experienced fever again and an increase of biological inflammatory markers (grey zone, Figure 1). On day 25, after 5 injections of L-aspa (day 10 injection was not performed), hepatic enzyme levels significantly increased, showing cholestasis and to a lesser extend cytolysis (AST level at 168 IU/l, ALT at 148 IU/l, γ GT at 735 IU/l, APL at 148 IU/l, and TBIL at 128 μ mol/l). Another L-aspa injection was done on day 26, but the last one was not prescribed, as hepatic enzymes still increased. At that time, bone marrow aspirate showed achievement of complete remission. A large and systematic diagnosis strategy, including screening for

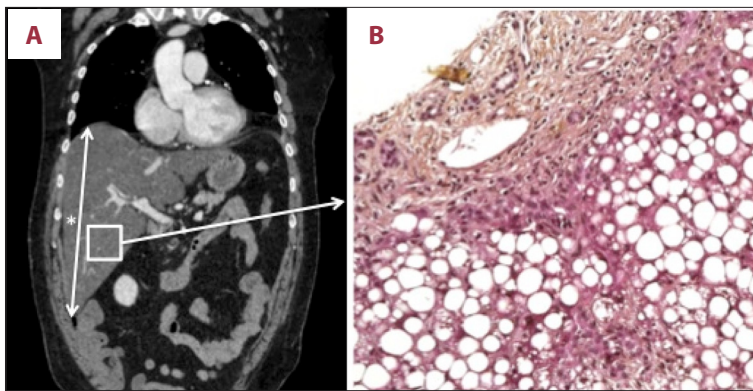


Figure 2. (A) Computed tomography revealed hepatomegaly (*=hepatic arrow 22.2 cm) with major homogeneous steatosis. (B) Liver biopsy showed a diffuse lobular steatosis >50% of hepatocytes, with moderate hepatocyte necrosis, and mild inflammatory lobular and portal infiltration (HE $\times 10$).

viral infections (HBV, HCV, HAV, HEV, VZV, EBV, HSV, CMV, HIV, PVB19), and autoantibodies (anti nuclear, anti-neutrophil cytoplasmic, anti-mitochondria, anti-actin, anti-endoplasmic reticulum, anti-LKM1, and anti-GP210), was negative. Lipid profile only revealed an isolated hypertriglyceridemia (3.3 g/l). A second abdominal echography, and contrast computed tomography were performed, this time showing homogeneous hepatomegaly (hepatic arrow at 22.2 cm) with signs of major steatosis (Cf Figure 2A), and no sign of biliary obstruction or vascular abnormality. There were no hypodense nodular lesions. Bacterial and mycological blood cultures remained negative. A liver biopsy was performed at J34 (AST level at 179 IU/l, ALT at 266 IU/l, γ GT at 1425 IU/l, APL at 266 IU/l, and TBIL at 124 μ mol/l) by transjugular route. Pathology examination revealed macro- and microvesicular severe steatosis, affecting more than 50% of hepatocytes, with a moderate hepatocyte necrosis, and a mild portal and lobular inflammatory infiltration, including rare neutrophils (Figure 2B). Gomori-Grocott coloration was negative, and bacterial assessment remained sterile. All running treatments were stopped except acetyl salicylic acid because of the cardiac history.

Concomitantly, the patient presented a cough related to an interstitial pneumonia. *Pneumocystis jirovecii* infection was documented by PCR from bronchoalveolar lavage products. Arterial oxygen pressure was at 58 mmHg (without oxygen therapy). A curative treatment, combining atovaquone with systemic corticosteroids (0.5 mg/kg/day for 10 days), was initiated on day 36 (CRP level at 50 mg/l, AST at 200 IU/l, ALT at 292 IU/l, γ GT at 1755 IU/l, APL at 260 IU/l, and TBIL at 123 μ mol/l), yielding to a rapid decrease of the inflammatory syndrome. Antioxidant therapy with N-acetyl cysteine was concomitantly started. Prothrombin time was maintained at about 60–70% with daily intravenous vitamin K supplementation.

No clinical or biological evidence of hepatic failure was observed. Finally, biological cholestasis and hepatic cytolysis progressively decreased. A control abdominal ultrasound also revealed the regression of hepatomegaly. On day 68, hepatic tests tended to be in normal range (AST level at 43 IU/l, ALT at 79 IU/l, γ GT

at 80 IU/l, APL at 47 IU/l, and TBIL at 19 μ mol/l). Consolidation chemotherapy could therefore be administered, but L-aspa was definitively banned. After 3 months, hepatic test results were in normal range, except for γ GT level at 85 IU/l. Intensification by allogeneic stem cell transplantation is ongoing.

Discussion

L-aspa is regarded as a major agent for the treatment of ALL [1]. However, physicians treating ALL are appropriately concerned about L-aspa toxicity and tolerability in the adult population. The majority of toxic effects of L-aspa are related to immune reactions to the bacterial protein [9,10]. Secondly, the effects of asparaginase and glutaminase depletion occurred, with subsequent inhibition of protein synthesis in organs such as liver and pancreas. Thrombosis or hemorrhages due to coagulation disturbances, acute pancreatitis, and liver dysfunctions can frequently be observed [6]. However, severe steatohepatitis, another life-threatening toxicity, has rarely been reported [7 8]. In an old retrospective study of Oettgen et al. [11], fatty metamorphosis of the liver (sometimes with necrosis of hepatocytes) was recorded in 40/55 systematic autopsies of patients treated with L-aspa.

The present case reports a febrile hepatomegaly occurring during neutrophil recovery, after intensive chemotherapy for ALL. The main clinical differential diagnosis was hepatosplenic candidosis. Risk factors for invasive candidosis are well known and include prior digestive colonization in the setting of a prolonged neutropenia combined with the use of broad-spectrum antibiotics [12]. Computed tomography and abdominal ultrasound may be negative [13]. Liver biopsy should be performed to eliminate differential diagnosis [14], as illustrated in the current case. Elimination of other non toxic origins was compatible with an iatrogenic disorder. Vincristine and cyclophosphamide have been rarely associated with liver abnormalities. Furthermore, microvesicular steatosis has never been reported with these agents [15,16]. Hepatomegaly, pathology showing severe macro- and microvesicular steatosis with mild necrosis,

as previously described [7,8,11], and rapid improvement after L-aspa discontinuation were in favor of L-aspa specific toxicity. In this case, fever and elevated C reactive protein were probably due to the concomitant *Pneumocystis*.

Pre-existing metabolic comorbidities may represent predisposing factors for the occurrence of L-aspa toxicity. With the exception of 2 pediatric cases reported by Sahoo, most of the patients experiencing L-aspa-associated steatosis were aged 50 years or older [7,8]. Prevalence of non-alcoholic fatty liver disease (NAFLD) in the 50-year-old population is currently increasing in Western countries, as recently highlighted by Williams et al. Prevalence was estimated at 46% in 400 volunteers (mean age 54.6 years) [17]. We therefore hypothesized that NAFLD could be an important predisposing factor for L-aspa toxicity in the reported case. Data on comorbidities are not available in the other published cases. However, 1 patient had, as in our case, a prior cardiovascular disease, which has been combined with obesity, frequently associated with NAFLD [18]. The number of doses of L-aspa to be administered is therefore questionable in case of pre-existing liver test abnormalities, which should, at a minimum, be carefully monitored.

Very few data are currently available regarding mechanisms involved in L-aspa-associated steatohepatitis. In a rabbit model, fatty vacuolization of hepatocytes was demonstrated after L-aspa administration. However, no direct correlations were observed with dose levels or L-aspa-specific activity [19]. Recently, Wilson et al demonstrated the involvement of general control non-derepressible kinase 2 (GCN2) in amino acid stress response during L-aspa therapy and, as a consequence, its ability to limit hepatic toxicity. Patients with GCN2 deficiency could have higher risk of liver steatosis and inflammation [20].

Regarding non-alcoholic fatty liver disease treatment, antioxidant therapy by N-acetyl cysteine has to be considered. Its beneficial effect in the management of other various liver diseases, such as alcoholic hepatitis [21], acute liver failure complicating dengue viral infection [22], non-acetaminophen acute liver failure [23], or acetaminophen acute liver failure [24], is now well established.

Pneumocystis was treated with atovaquone, known for being less toxic than trimethoprim/sulfamethoxazole, and systemic steroid administration justified by *Pneumocystis*-induced hypoxemia. Concomitant steroid administration has been shown to dramatically decrease the clinical symptoms of allergy, when compared with patients receiving L-aspa only [9,10]. Clinical

presentation and pathology were very similar to those observed in acute alcoholic hepatitis, for which corticosteroids can improve outcome in the most severe cases [25,26]. Importantly, no published data have previously supported a benefit of corticosteroids in L-aspa-associated liver disease; thus, no recommendation could be made. However, we could not rule out that this treatment may be responsive of the favorable outcome.

Because of the risk of fatal liver failure, L-aspa was discontinued and definitively avoided in further chemotherapy courses. Other L-aspa presentations and formulations should also be avoided because they can impair the hematological prognosis due to the major role of this agent in the treatment of ALL. It has been shown that the repetition of L-aspa doses is associated with better prognosis [27]. In the current case, allogeneic stem cell transplantation has been scheduled because of high-risk features of ALL (Ikaros deletion and MLL rearrangement). Special hepatic test monitoring is required with regard to high risk of hepatic failure related to toxicity of agents used during the conditioning regimen, and/or severe hepatic graft-versus-host disease, and/or hepatic veno-occlusive disease.

Conclusions

This case illustrates the necessity to carefully monitor liver function in patients receiving chemotherapy including repeated doses of L-aspa, most particularly in patients with evidence of pre-existing liver disease or potential risk factors. A systematic and invasive diagnosis strategy should be systematically performed in case of liver abnormalities. A hepatic biopsy should promptly be performed to exclude differential diagnosis such as septic hepatitis in patients with prolonged neutropenia. There is no consensus regarding treatment. As soon as possible, L-aspa and other hepatotoxic drugs must be stopped and antioxidant therapy should be initiated. In our case, the concomitant administration of systemic corticosteroids, because of concomitant *Pneumocystis* with hypoxemia, may have been a beneficial factor in the rapid recovery. Prospective monitoring of liver enzymes during L-aspa therapy should be performed, as well as a better characterization of risks factors, determination of potential mechanisms, and useful treatment of this life-threatening toxicity.

Disclosure

All authors report no conflict of interest.

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